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Effects of Some Complexing Agents and Modified Bovine Serum Albumins on Lecithin-Cholesterol Acyltransferase Inhibition caused by Cu^{2+} or Hg^{2+} Ions

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Lecithin-cholesterol acyltransferase [EC 2.3.1.43] (LCAT) activity in human plasma was inhibited by the addition of 1×10^{-3} M Cu^{2+} or Hg^{2+} ions as well as Zn^{2+} or Cd^{2+} ions. In contrast, the acyltransferase activity was stimulated by the addition of 1×10^{-3} M cysteine, mercaptoethanol, dimercaptosuccinate, penicillamine, thioglucose or thiourea but not by the addition of 1×10^{-3} M histidine or ethylenediaminetetraacetic acid (EDTA). The most effective stimulation was obtained by the addition of mercaptoethanol.

On the other hand, dimercaptoethanol among the sulfhydryl agents mentioned above effectively reversed the acyltransferase inhibition caused by Zn^{2+} or Cd^{2+} ions but not that caused by Cu^{2+} or Hg^{2+} ions. EDTA completely reversed the acyltransferase inhibition caused by Cu^{2+} or Cd^{2+} ions but not that caused by Hg^{2+} ions. Histidine slightly reversed the acyltransferase inhibition caused by Cu^{2+}, Cd^{2+} or Zn^{2+} ions. Furthermore, native, acetylated or succinylated albumin effectively reversed the acyltransferase inhibition caused by Zn^{2+} or Cd^{2+} ions but not that caused by Cu^{2+} or Hg^{2+} ions.

Keywords——LCAT; cysteine; mercaptoethanol; dimercaptosuccinate; penicillamine; thioglucose; thiourea; modified albumin; heavy metal ion

It has been reported that the plasma cholesterol content of rats which have ingested copper and zinc is increased by increase in the zinc/copper ratio[1,2] and that a low level of dietary cadmium induces hypertension in rats.[3] Increase of plasma cholesterol content and hypertension are well known to be important factors in the prediction of risk in human atherosclerosis .On the other hand, it is known that complexing agents such as EDTA, penicillamine, 2,3-dimercaptopropanol and mercaptoethanol are effective for the elimination of heavy metal ions accumulated in animal tissues.[4,5] Therefore, it is of interest to investigated the effects of heavy metal ions on cholesterol metabolism in human plasma and of complexing agents on plasma cholesterol metabolism in the presence of heavy metal ions. We have previously reported that LCAT, which catalyzes the formation of cholesterol ester from lecithin and cholesterol in human plasma,[6] is inhibited by the addition of Zn^{2+} or Cd^{2+} ions and also that the acyltransferase inhibition caused by Zn^{2+} or Cd^{2+} ions is partially or completely reversed by the addition of EDTA or modified (acetylated or succinylated) bovine serum albumins but not by the addition of citrate or cysteine.[7,8]

In this paper, the effects of some complexing agents (cysteine, mercaptoethanol, dimercaptosuccinate, penicillamine, thioglucose, thiourea, histidine or EDTA) and modified (acetylated or succinylated) bovine serum albumins on the acyltransferase reaction in human plasma in the presence of Cu^{2+} or Hg^{2+} ions were compared with those in the presence of Zn^{2+} or Cd^{2+} ions.

Experimental

Materials——^{3}H-Cholesterol was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.) and purified as described previously.[9] Bovine serum albumin was obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Lecithin was prepared from egg yolk by the method of Faure[10] and purified by silicic acid column chromatography. The other chemicals used here were of reagent grade, purchesed from

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Kanto Chemical Co. (Tokyo, Japan).

Preparation of Modified Albumin——Acetylated and succinylated albumins were prepared by the procedure of Kidwai *et al.*[11] as described previously.[8] The extent of chemical modification of bovine serum albumin was determined by means of the ninhydrin reaction as described by Sluyterman and De Graaf.[12]

Measurement of Viscosity——The viscosities of native and modified albumins were measured in phosphate buffer (pH 7.0, ionic strength 0.1) by using an Ostwald viscometer with a time of 127.4 s for 2 ml of water at 25°C. The intrinsic viscosity (η) was computed as described previously.[8]

Spectral Measurement——The circular dichroism absorptions of native and modified albumins were measured in phosphate buffer (pH 7.0, ionic strength 0.1) with a Nihon Bunko circular dichroism spectropolarimeter (model J-40A-S) as described previously.[8]

Preparation of Substrate Dispersion——Sonicated dispersions of lecithin–cholesterol mixtures were prepared as described previously.[13] The contents of lipid phosphorus and cholesterol in substrate dispersions were determined by the procedure described by Muesing and Nishida.[14]

Preparation of Human Plasma——Human plasma was obtained from outdated human blood containing 0.15 volume of anticoagulant solution (citric acid, sodium citrate and glucose) by centrifugation and was then dialyzed against Tris-HCl buffer, pH 7.0, ionic strength 0.1. The resulting plasma was diluted with Tris-HCl buffer to give a protein content of 65 mg/ml. The protein content was determined by the procedure described by Lowry *et al.*[15] using crystalline bovine serum albumin as a standard.

Enzyme Assay——The incubation mixture contained 0.1 ml of the substrate dispersion and 0.2 ml of human plasma. The final volume was adjusted to 0.5 ml with Tris-HCl buffer, pH 7.0. The concentrations of various materials added to the incubation medium are described elsewhere in the text. The samples were placed in 15 ml screw-capped tubes, which were flushed with N_2, sealed and incubated for 3 h at 37°C with constant shaking. After incubation, extraction and separation of lipids and measurement of radioactivity were carried out as described previously.[9]

Results and Discussion

As shown in Table I, the acyltransferase activity was inhibited by the addition of 1×10^{-3} M Cu^{2+} or Hg^{2+} ions as well as Zn^{2+} or Cd^{2+} ions. The inhibitory potencies of Cu^{2+} and Hg^{2+} ions on the acyltransferase activity were almost the same as that of Cd^{2+} ions. On the other hand, the acyltransferase activity was stimulated by the addition of 1×10^{-3} M cysteine,

TABLE I. Effects of Some Complexing Agents on LCAT Activity in Human Plasma in the Presence or Absence of 1×10^{-3} M $ZnCl_2$, $CdCl_2$, $CuCl_2$ or $HgCl_2$

Expt. No.	Heavy metal ions	None	Cysteine	Mercapto-ethanol	Dimercapto-succinate	Penicill-amine	Thiourea	Thio-glucose	Histidine	EDTA
I	None	5.9(100)	—	—	7.6(100)	7.8(100)	7.1(100)	8.2(100)	6.1(100)	—
	Zn^{2+}	2.7(46)	—	—	5.7(75)	2.3(29)	3.0(42)	3.4(41)	3.6(59)	—
	Cd^{2+}	2.1(36)	—	—	7.6(100)	4.6(59)	2.7(38)	3.1(38)	2.8(46)	—
	Cu^{2+}	2.0(34)	—	—	2.1(28)	2.4(31)	3.0(42)	2.2(27)	2.8(46)	—
II	None	4.4(100)	6.9(100)	8.1(100)	5.4(100)	6.2(100)	5.1(100)	6.7(100)	4.4(100)	—
	Zn^{2+}	2.5(56)	2.6(38)	3.7(46)	4.6(85)	2.0(32)	2.9(57)	3.4(51)	3.0(68)	—
	Cd^{2+}	1.8(40)	3.3(48)	4.1(51)	5.4(100)	3.4(55)	2.2(43)	2.4(36)	2.3(52)	—
	Cu^{2+}	1.7(39)	1.8(26)	1.9(23)	2.0(37)	1.9(31)	2.8(54)	1.7(25)	2.3(52)	—
III	None	3.4(100)	5.1(100)	7.2(100)	5.1(100)	5.5(100)	—	—	3.4(100)	3.4(100)
	Cd^{2+}	1.4(41)	3.0(59)	4.0(56)	4.4(86)	3.0(55)	—	—	1.9(56)	3.4(100)
	Cu^{2+}	1.4(41)	1.4(27)	2.3(32)	2.1(41)	1.3(24)	—	—	1.8(53)	3.3(97)
	Hg^{2+}	1.0(29)	1.0(17)	1.2(17)	1.6(31)	1.1(20)	—	—	1.2(35)	1.0(29)
IV	None	5.2(100)	—	—	6.2(100)	—	—	—	5.4(100)	5.4(100)
	Cd^{2+}	1.8(35)	—	—	6.0(97)	—	—	—	2.8(52)	5.2(96)
	Cu^{2+}	1.6(31)	—	—	1.6(26)	—	—	—	2.6(48)	5.0(93)
	Hg^{2+}	1.5(29)	—	—	1.5(24)	—	—	—	1.9(35)	1.6(30)

The incubation mixture contained 0.1 ml of substrate dispersion (lecithin/cholesterol molar ratios of 5.3, 5.4, 5.0 and 5.6 for Experiments I, II, III and IV, respectively), 0.2 ml of human plasma, 1×10^{-3} M heavy metal ions and 1×10^{-3} M complexing agent. The final volume was adjusted to 0.5 ml with Tris-HCl buffer, pH 7.0. Incubation was carried out at 37°C for 3 h. The amounts of radioactivity and free cholesterol in one ml of the dispersions with lecithin/cholesterol molar ratios of 5.3, 5.4, 5.0 and 5.6 were 1 μCi/0.65 μmol, 1 μCi/0.63 μmol, 1 μCi/0.59 μmol and 1 μCi/0.64 μmol, respectively. The values in parentheses are percentages of the values obtained in the absence of heavy metal ions (taken as 100%).

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mercaptoethanol, dimercaptosuccinate, penicillamine, thioglucose or thiourea but not by the addition of 1×10^{-3} M histidine or EDTA. The most effective stimulation of the acyltransferase activity was obtained by the addition of mercaptoethanol. The stimulatory effects of sulfhydryl agents mentioned above on the acyltransferase activity may be mainly due to a protecting action on the sulfhydryl groups in the acyltransferase, because the acyltransferase in human plasma requires one or more sulfhydryl groups for its normal catalytic activity.[16] However, Verdery has recently reported that mercaptoethanol and dithiothreitol stimulate the acyltransferase activity while cysteine and reduced glutathione inhibit the acyltransferase activity.[17] At the same time, he suggested that, from the contrasting effects of different reducing agents on the acyltransferase activity, the role of sulfhydryl groups in the acyltransferase is complex.[17] In any event, under the present experimental conditions, we could not observe the inhibitory action of cysteine on the acyltransferase activity. In addition, thiourea, which is not a sulfhydryl agent, slightly stimulated the acyltransferase activity. Although the mechanism of stimulatory action of thiourea on the acyltransferase activity is not clear, the stimulatory effect of thiourea is very different from the effect of urea, which strongly inhibits the acyltransferase activity.[18]

On the other hand, the acyltransferase inhibition caused by Zn^{2+} ions was effectively reversed by the addition of dimercaptosuccinate and was slightly reversed by the addition of histidine. However, cysteine, mercaptoethanol, penicillamine, thioglucose or thiourea was not effective for the reversal of the acyltransferase inhibition caused by Zn^{2+} ions. Similarly, the acyltransferase inhibition caused by Cd^{2+} ions was almost completely reversed by the addition of dimercaptosuccinate or EDTA and was slightly reversed by the addition of cysteine, mercaptoethanol, penicillamine or histidine. Also, the acyltransferase inhibition caused by Cu^{2+} ions was completely reversed by the addition of EDTA and was slightly reversed by the addition of histidine. However, sulfhydryl agents tested here were not effective for the reversal of the acyltransferase inhibition caused by Cu^{2+} ions. Furthermore, the acyltransferase inhibition caused by Hg^{2+} ions was little affected by the addition of sulfhydryl agents tested here, thiourea or histidine. In addition, although the stability constant (k) of Hg^{2+}-EDTA complex (k=21.80, ionic strength 0.1, 20°C) is higher than those of Zn^{2+}-EDTA (k=16.50), Cd^{2+}-EDTA (k=16.46) and Cu^{2+}-EDTA (k=18.80) complexes, the inhibitory action of Hg^{2+} ions on the acyltransferase activity was not reversed by the addition of EDTA.

Heavy metal ions such as Hg^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} ions may interact mainly with sulfhydryl groups (cysteine residues), imidazole groups (histidine residues) and carboxyl groups of human plasma proteins.[19] Therefore, the results obtained here suggest that the Hg^{2+}, Cu^{2+}, Cd^{2+} or Zn^{2+} ions may bind to these groups of plasma proteins more strongly than to cysteine, mercaptoethanol, penicillamine, thioglucose, thiourea or histidine, and that the Cd^{2+} or Zn^{2+} ions may bind to these groups of plasma proteins more loosely than to dimercaptosuccinate. In addition, Cu^{2+} ions as well as Cd^{2+} ions may bind to plasma proteins more loosely than to EDTA while Hg^{2+} ions may bind to plasma proteins more strongly than to EDTA.

We next investigated whether the acyltransferase inhibition caused by Cu^{2+} or Hg^{2+} ions as well as Cd^{2+} and Zn^{2+} ions is reversed by the addition of native, acetylated or succinylated albumin. As shown in Table II, the acyltransferase inhibition caused by Cd^{2+} or Zn^{2+} ions was partially or completely reversed by the addition of native, 34%-acetylated or 45%-succinylated albumin. However, the acyltransferase inhibition caused by Cu^{2+} or Hg^{2+} ions was little affected by the addition of native, 34%-acetylated or 45%-succinylated albumin. These results suggest that the interaction of Cu^{2+} or Hg^{2+} ions with plasma proteins such as the enzyme (LCAT) or high density lipoprotein ($1.063<d<1.210$ g/cm^3), which is a cofactor lipoprotein for the acyltransferase reaction,[20] may be stronger than that of Cu^{2+} or Hg^{2+} ions with native, acetylated or succinylated albumin and also that the Cu^{2+} or Hg^{2+} ions bound

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Table II. Effects of Native and Modified Albumins on LCAT Activity in Human Plasma in the Presence or Absence of 1×10^{-3} M Heavy Metal Ions

Expt. No.	Addition and physical properties	Cholesterol esterified (%) None	Albumin	34%-acetylated albumin	45%-succinylated albumin
I	None	5.2(100)	5.6(100)	5.5(100)	5.3(100)
	Cd^{2+}	1.8(35)	4.0(71)	4.4(80)	4.2(79)
	Cu^{2+}	1.6(31)	1.8(32)	2.0(36)	1.9(36)
	Hg^{2+}	1.5(29)	1.6(29)	1.7(31)	1.6(30)
II	None	9.2(100)	10.8(100)	10.7(100)	10.0(100)
	Cd^{2+}	2.3(25)	7.4(69)	8.2(77)	7.8(78)
	Cu^{2+}	1.7(18)	2.0(19)	3.2(30)	2.2(22)
	Hg^{2+}	1.6(17)	2.0(19)	2.2(21)	1.8(18)
III	None	4.5(100)	5.1(100)	4.6(100)	4.9(100)
	Zn^{2+}	1.9(42)	4.0(78)	4.5(99)	3.8(78)
	Cd^{2+}	1.5(33)	3.2(63)	4.1(89)	3.5(71)
	Cu^{2+}	1.2(27)	1.2(24)	1.3(28)	1.0(20)
	Hg^{2+}	0.8(18)	1.0(20)	0.9(20)	0.9(18)
	Intrinsic viscosity (ml/g, η)		3.4	3.7	4.0
	$-[\theta]_{208} \times 10^{-6}$ (deg·cm²/dmol)		12.6	12.8	12.6
	α-Helix content (%)		58.0	59.1	58.0

The incubation mixture contained 0.1 ml of substrate dispersion (lecithin/cholesterol molar ratios of 5.3, 5.6 and 6.0 for Experiments I, II and III, respectively), 0.2 ml of human plasma and 20 mg of various albumins. The other incubation conditions were the same as in Table I. The amounts of radioactivity and free cholesterol in one ml of the dispersions with lecithin/cholesterol molar ratios of 5.3, 5.6 and 6.0 were 1 μCi/0.65 μmol, 1 μCi/0.77 μmol and 1 μCi/0.63 μmol, respectively. The values in parentheses are percentages of the values obtained in the absence of heavy metal ions (taken as 100%).

to plasma proteins may bind to amino acid residues other than those carrying carboxyl groups in plasma proteins.

In addition, the secondary structures of the modified albumins used here were little altered as compared with that of native albumin, as revealed by intrinsic viscosity and circular dichroism measurements. These results are compatible with our previous observations that the ordered secondary structure of bovine serum albumin was only slightly altered by 52%-acetylation or 48%-succinylation of its lysine residues.[8]

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